ERNDIM CSF neurotransmitter pilot scheme: Review of the first year

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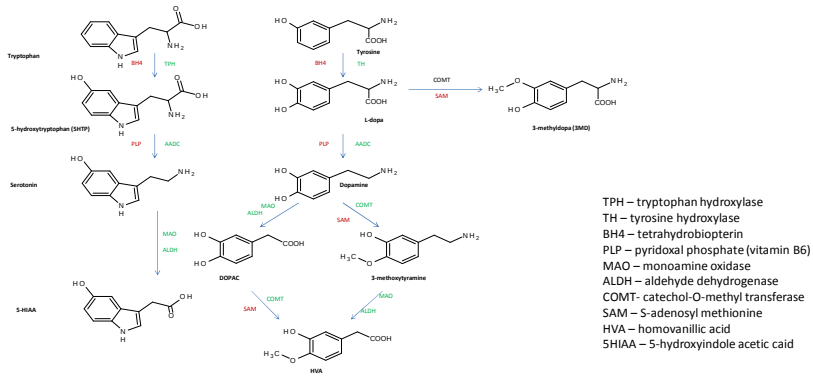
Background/objectives: This was the first year of the ERNDIM CSF neurotransmitter pilot scheme. The scheme was set up to monitor the analytical quality and interpretation of the quantitative assay of monoamine metabolites in CSF.



TPH – tryptophan hydroxylase
TH – tyrosine hydroxylase
BH4 – tetrahydrobiopterin
PLP – pyridoxal phosphate (vitamin B6)
MAO – monoamine oxidase
ALDH – aldehyde dehydrogenase
COMT- catechol-O-methyl transferase
SAM – S-adenosyl methionine
HVA – homovanillic acid
5HIAA – 5-hydroxyindole acetic caid

Figure 1: Dopamine and serotonin metabolic pathways

Overview of scheme:
- 30+ laboratories applied to participate, although limited sample material meant the pilot scheme had to be restricted to 19 participants
- 2 labs measured by LC-MS, the rest by HPLC with electrochemical detection
- 8 samples (4 samples in duplicate) were sent to participating laboratories.

Sample preparation and analysis/interpretation:
- The samples were made by pooling, diluting and/or spiking 'control' CSF.
- Participating labs were asked to quantify 4 metabolites (Homovanillic acid (HVA), 5-hydroxyindole acetic acid (5HIAA), 3-methyl dopa (3MD) and 5-hydroxytryptophan (5HTP).
- There was also an multiple choice interpretation question – this was included as laboratories around the world have different reference ranges for the monoamine neurotransmitter metabolites, mainly dependent on the CSF fraction they use – there is a gradient within the CSF and later fractions contain higher concentrations of the monoamine metabolites. Therefore this multiple choice was included to see if interpretation differed depending on reference ranges.

The Samples:
1 'AADC deficiency' – sample B; 1 dopamine transporter defect – sample A;
2 'normal' CSFS – samples C and D

Results:
- 2 labs measured by LC-MS, the rest by HPLC with electrochemical detection
- 15 or 16 labs out of the 19 consistently returned results
- 5HIAA and HVA results were generally consistent between labs and between duplicate samples (CV ~10%)
- 3MD and 5HTP were much more variable between labs and between duplicates (13-63%). Mean values were also lower on repeat duplicates – degradation on storage? This will be investigated in more detail.

Duplicate samples - amounts in nmol/L	A	B	C	D
3MD	49	341	70	82
5HIAA	97	87	139	154
5HTP	13	111	22	25
HVA	1028	175	453	347
Ratio	10.6	2	3.3	2.3

Table 1: Metabolite concentrations measured in each sample by co-ordinating laboratory prior to lyophilisation
NB These values are higher than final concentrations in the samples sent to participating laboratories as the sample was split into more aliquots than originally intended.

Results from ERNDIM pilot scheme for duplicate samples		A1	A2	B1	B2	C1	C2	D1	D2
3MD	Mean	41.9	39.9	247	211	51.8	40	65.7	53.9
	SD	11.4	15.7	65.2	79.6	14.8	16.3	23.6	16.1
	CV	27.2	39.3	26.4	37.7	28.6	40.8	35.9	29.9
5HIAA	Mean	90.6	85.4	65	61.6	120	103	137	138
	SD	11.1	6.67	8.15	4.74	25.6	8.01	10.4	12.6
	CV	12.3	7.8	12.5	7.7	21.3	7.8	7.6	9.1
5HTP	Mean	12.1	11.0	74.9	54.2	14.7	12.6	16.6	17.4
	SD	2.97	1.48	17	34.2	5.93	4.35	4.08	7.78
	CV	24.5	13.5	22.7	63.1	40.3	34.5	24.6	44.7
HVA	Mean	914	896	142	142	354	333	305	302
	SD	74.1	62.6	18.5	6.82	45.2	16.3	17	13.2
	CV	8.1	7.0	13.0	4.8	12.8	4.9	5.6	4.4
Ratio	Mean	10.1	10.6	2.1	2.3	3.1	3.3	2.3	2.2
	SD	0.82	1.26	0.33	0.15	0.45	0.32	0.22	0.25
	CV	8.1	11.9	15.7	6.4	14.5	9.6	9.8	11.4

Table 2: Results from ERNDIM pilot scheme for duplicate samples

Conclusions:
- The first year of the scheme has worked well and 5HIAA and HVA results between laboratories are consistent.
- Over 85% of interpretive comments were correct (at least 14/16 laboratories correct).
- The problems with variation in 3MD and 5HTP results will be investigated and hopefully solved by using an artificial CSF in this years scheme. A preliminary study suggests that all the metabolites are stable in this matrix both before and after lyophilisation.